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Indigenous Biopreparations and Biocontrol Agents for Growth and Disease Management in Black Pepper Nursery

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Abstract

The present study was carried out at Agricultural Research Station, Thiruvalla, Kerala, India during December, 2020–July, 2021 to evaluate the efficacy of native biocontrol agents and fermented organic preparations viz; fish-jaggery extract, egg-lemon juice extract and jeevamrutha on growth enhancement and suppression of *Phytophthora* infection in black pepper nursery. The plants were challenge inoculated with *Phytophthora capsici* at the collar region. The plant growth parameters were recorded before and one month after treatment application. Results showed that a considerable increase in plant height (49.30%) and number of leaves (57.89%) with the application of bacterial antagonists A1 and S2 respectively. Application of fish-jaggery extract increased the shoot girth by 29.6%. Soil application of *Trichoderma* combined with spraying of bacterial antagonists A1 and S2 also increased the shoot girth considerably. Application of jeevamrutha increased the total leaf area by 49.98% and chlorophyll contents viz. chlorophyll a, chlorophyll b and total chlorophyll was also maximum with this application. *Phytophthora* infection at collar region was not observed in plants treated with potassium phosphonate and fish-jaggery extract. Application of egg-lemon juice extract, jeevamrutha and bioagents also recorded less infection on collar region. The study indicated the efficacy of fermented organic fertilizers and bioagents in improving plant growth and also in reducing pathogen infection at nursery stage. Hence a combination of native antagonistic microflora and indigenous biopreparations could be effectively utilized in commercial production of healthy planting materials in black pepper nurseries.

Keywords: Black pepper, biocontrol, biopreparations, disease, growth

1. Introduction

Black pepper (*Piper nigrum* L.), the 'King of Spices' has been cultivated in India since ancient times and it is the most important spice crop in trade all over the world. Despite the land of origin, production and productivity of black pepper in India has declined recently owing to Reduction in area, poor cultivation practices, new emerging diseases etc (Verma et al., 2023). The availability of quality planting material is a major constraint in increasing the area, production and productivity of black pepper. The propagation of black pepper is mainly through rooted cuttings and layers. In nurseries, the plantlets are prone to attack by a number of pests and diseases. Phytophthora rot is a major nursery disease which causes high mortality of cuttings. At times this fungus is carried from diseased areas to healthy areas through planting material. Integrated approach involving cultural, biological and chemical methods are reported for successful management of this disease (Anandaraj, 2000; Bhai et al., 2017; Hegde and Jahagirdar, 2017). Replacement of synthetic fungicides by natural and safe plant protectants which can interfere with the fungal pathogenicity factors has become very important in plant disease management (Karthika et al., 2017; Ayilara et al., 2023). Biological control using rhizosphere microorganisms has now gained importance as feasible and eco-friendly approach for sustainable agriculture (Jiao et al., 2021; El-Saadony et al., 2022). Many potential biocontrol agents have been identified for managing Phytophthora rot diseases (Segarra et al., 2013; Rini and Remya, 2020; Sonavane and Sriram, 2021). These organisms have ability to colonize plants and directly suppress plant pathogens by the production of antibiotics, siderophore production, competition with pathogens for resources and induction of systemic acquired host resistance. These agents are also known for their effect on plant growth enhancement by increased nutrient uptake and utilization, better photosynthetic activity and resistance to both biotic and abiotic stresses (Calvo et al., 2014; Colla et al., 2015; Van Oosten et al., 2017; Rouphael et al., 2017; Salomon et al., 2017; Etesami, 2018; Tariq et al., 2020). They promote root and shoot growth by secreting hormone-like compounds, nodule formation and mycorrhiza

establishment (Michał et al., 2019; Sharma, 2021). In recent years, application of many indigenous organic preparations has been introduced to modern agriculture for producing safe and good quality agriculture products (Swaminathan et al., 2007). These bioproducts possess antifungal potential, induce resistance to pests and diseases, Stimulates plant growth, enhance plant nutrient uptake and improve the keeping quality of the produce. They can be composed of plant growth promoting bacteria and fungi, plant extracts or animals-derived compounds (Natarajan, 2002; Du Jardin, 2015; Ruzzi and Aroca, 2015; Wagner and Hetman, 2016). Liquid organic manures such as panchagavya, beejamruth, jeevamruth, vermiwash, humic acid, sea weed extract, fish amino, egg amino etc could be considered as a good plant and soil tonic which effectively resist pest and diseases, improve plant growth (Weinert et al., 2014; Varshini and Jayanthi, 2020; Goveanthan et al., 2020; Meyyappan et al., 2021; Nongtdu et al., 2022) and restore the vitality of the soil (Michalak et al., 2016). Plants drenched with jeevamruth consistently produce finer leaves and denser canopy. Aulakh et al. (2013) revealed that jeevamruth application significantly increased the microbial population of soil. Sajeena et al. (2015) reported the inhibitory effect of fermented egg-lemon juice extract as well as fish amino acid on growth of *R solani* in amaranthus. With this background an attempt was made to evaluate some native isolates of antagonistic microorganisms and farm made fermented organic preparations on growth and disease management in black pepper cuttings in nursery.

2. Materials and Methods

2.1. Isolation and in vitro evaluation of antagonistic microorganisms

The present investigation was carried out during December, 2020-July, 2021 at Agricultural Research Station, Thiruvalla, Pathanamthitta District, Kerala, India located at latitude of 9.21°24' N and longitude of 76.33°41' E. Native antagonistic bacteria and fungi were isolated from the soil samples collected from the rhizosphere of healthy black pepper vines following dilution plate technique. *In vitro* screening was done using dual culture technique (Raupach and Kloepper, 1998) to identify isolates antagonistic to *Phytophthora capsici*, the foot rot pathogen of black pepper and the best isolates were selected, purified and maintained for further studies.

2.2. Preparation of fermented organic plant growth stimulants

Three organic preparations viz; jeevamrutha, fish-jaggery extract and egg-lemon juice extract were chosen for the study. For preparing jeevamrutha, procedure given by Chadha et al., (2012) was followed where, cow dung (1 kg), cow urine (1 L), pulse flour (green gram-200 g), jaggery (200 g) and fertilizer free fertile top soil (100 g) were used for 10 l jeevamrutha. All ingredients were mixed well in a container, covered with a gunny bag and kept in shade for seven days. The mixture was stirred three times daily using wooden ladle. After seven days the mixture was strained through a muslin cloth and kept for study.

Fish-jaggery extract was prepared as per the procedure described by Weinert et al. (2014). Sardine fish and jaggery at 1:1(w/w) ratio was used for the preparation. Fish cut into small pieces (one inch size) were filled in container in layers by adding powdered jaggery in between each layer. The container was kept closed and undisturbed for five days after which the contents were stirred once daily using wooden ladle upto 21 days for proper fermentation. It was then filtered through a muslin cloth and stored for use in the study.

Preparation of egg-lemon juice extract was done by following procedure given by Sajeena et al. (2016). Hen eggs (5 number) were kept immersed in lemon juice (10–15 number lemon) taken in a container, well closed and kept undisturbed for ten days. After ten days 250 g powdered jaggery was added. The eggs were mashed and mixed thoroughly with the jaggery and kept for ten more days, then filtered through muslin cloth and stored.

2.3. In vivo evaluation of biocontrol agents and fermented preparations under green house condition

The promising antagonists selected under dual culture technique and the fermented biopreparations were evaluated on three months old rooted cuttings of black pepper for their effect on growth enhancement and disease suppression. The experiment was laid out in completely randomized design with three replications. Talc based formulation of selected bacteria (2%), fish-jaggery extract (0.5%) and egg-lemon juice extract (0.5%) and jeevamrutha (10%) were sprayed on whole plant and drenched to root zone of rooted pepper cuttings grown in poly bags. A second round spray was given after 10 days. *Trichoderma* sp. grown in rice bran was applied to root zone at the rate of 5 g kg^{-1} soil. A challenge inoculation with the pathogen *P. capsici* was made at the collar region of the cuttings after three days of treatment application. Application with potassium phosphonate at 0.3% concentration was kept as chemical check and plants without any treatment served as control. The plant growth parameters viz., plant height, shoot girth, total number of leaves, total leaf area etc. were recorded before and one month after treatment application and percent increase was calculated. Chlorophyll content of the leaves was estimated following standard procedures (Sadasivam and Manickam, 1992) after one month of treatment application. Length of lesion at collar region was recorded for estimating stem infection. Statistical analysis was done as per Gomez and Gomez (1984).

3. Results and Discussion

Data showed that, among the different treatments, application of bacterium isolate A1 showed maximum increase in plant height of 49.30% as against 23.56% increase in control (Table 1). Application of potassium phosphonate resulted in 37.57% increase in plant height. The plants treated with

Table 1: Effect of biocontrol agents and fermented organic products on growth and disease suppression of rooted cutting in black pepper nursery

Treatments	Plant height (% increase)*	Shoot girth (% increase)*	Total number of leaves (% increase)*	Total leaf area (% increase)*	Chlorophyll content (mg g^{-1} tissue)			Collar infection (lesion length in cm)
					Chl.a	Chl.b	Total chl.	
Bacterium A1	49.3^{a}	21.35^{b}	52.38^{ab}	27.82^{d}	0.3^{ab}	0.26^{b}	0.56^{b}	2.33^{bc}
Bacterium S2	36.65^{b}	26.92^{a}	57.89^{a}	42.54^{b}	0.25^{de}	0.16^{d}	0.41^{d}	2.88^{bc}
Trichoderma sp.	21.14^{cd}	12.64^{c}	43.75^{d}	27.64^{d}	0.3^{ab}	0.22^{b}	0.53^{bc}	3.58^{cd}
A1+*Trichoderma* sp.	29.03^{bc}	28.21^{a}	39.41d^{e}	25.54^{d}	0.28^{bc}	0.22^{c}	0.5^{c}	2.10^{b}
S2+*Trichoderma* sp.	35.65^{b}	27.71^{a}	52.94^{ab}	38.65^{bc}	0.28^{bc}	0.25^{c}	0.52^{bc}	2.28^{bc}
Fish-Jaggery extract	18.88^{d}	29.63^{a}	31.58^{f}	29.99^{d}	0.22^{f}	0.13^{e}	0.34^{e}	0.00^{a}
Egg-lemon juice extract	17.79^{d}	13.25^{c}	50.00^{bc}	32.59^{cd}	0.27^{cd}	0.25^{b}	0.52^{bc}	0.43^{a}
Jeevamrutha	16.19^{d}	16.3^{c}	44.64^{cd}	49.98^{a}	0.32^{a}	0.29^{a}	0.61^{a}	1.10^{ab}
Potassium phosphonate	18.48^{d}	21.04^{b}	35.29^{ef}	28.77^{d}	0.24^{ef}	0.21^{c}	0.45^{d}	0.00^{a}
Control	23.56^{cd}	6.1^{d}	23.81^{g}	26.12^{d}	0.19^{g}	0.15^{de}	0.34^{e}	4.23^{de}
CD (p=0.05)	8.052	3.07	5.777	7.068	0.027	0.029	0.046	1.06

*% increase in one month period

bacterial antagonist S2 individually and in combination with *Trichoderma* sp. also recorded considerable increase in plant height with an increase of 36.65% and 35.65% respectively. The shoot girth at collar region was maximum increased in plants treated with fish-jaggery extract (29.63%). Next best treatments were application of bacterium A1+*Trichoderma* sp. (28.21%), bacterium S2+*Trichoderma* sp. (27.71%) and individual application of bacterium S2 (26.92%) while in control the increase observed was only 6.1%. Individual application of bacterium A1 (21.35%) also recorded significant increase in shoot girth.

Regarding the total number of leaves, plants treated with bacterial antagonist S2 recorded maximum increase of 57.89% followed by combined application of bacterium S2+*Trichoderma* sp. (52.92%) and A1 (52.38%) as against 23.81% increase in control. In case of total leaf area, application of jeevamrutha recorded highest increase (49.98%) followed by application of bacterium S2 (42.54%) whereas in control the increase in leaf area recorded was 26.12%.

Chlorophyll contents viz., chlorophyll a, chlorophyll b and total chlorophyll was maximum observed in plants treated with jeevamrutha with 0.32, 0.29 and 0.61 mg g^{-1} plant tissue. Application of bacterium A1 and S2 individually and in combination with *Trichoderma* sp. and egg-lemon juice extract also showed higher chlorophyll content than the control plants. In control plants, chlorophyll a, b and total chlorophyll recorded was 0.19, 0.15 and 0.34 mg g^{-1} plant tissue respectively.

The data on the effect of different treatments on *Phytophthora* infection at collar region showed considerable reduction in the disease when compared to control. *Phytophthora* infection at collar region was not observed in plants treated with potassium phosphonate and fish-jaggery extract. Application of egg-lemon juice extract and jeevamrutha also recorded less infection on collar and is statistically on par with the effect of potassium phosphonate. Spraying and drenching with bacterial antagonists A1 and S2 alone or in combination with *Trichoderma* sp. reduced the infection to a considerable level as indicated by smaller infection lesions as compared to control.

Several workers have reported the efficacy of biocontrol agents in enhancing plant growth in addition to their ability in reducing plant diseases (Manoranjitham et al*.,* 2000). In our study, application of native antagonistic microbial isolates improved the plant growth in terms of plant height, shoot girth, number of leaves and chlorophyll content besides reducing pathogenic infection. The interaction of rhizosphere microorganisms with the plant roots improves the plant growth by increased uptake of mineralised nutrients, vitamins and other growth hormones. This interaction also aids in inhibition of plant pathogens and provides stress tolerance under field conditions (Sindhu and Sharma, 2019). Dubeikovsky et al. (1993) stated that plant growth regulators like gibberllins, cytokinins and indole acetic acid (IAA) induced by the strains might have contributed for better plant growth and development. Application of bacterial antagonists in combination with *Trichoderma* resulted in increased growth and reduced infection. The results are in consensus with the findings of Anith and Manomohandas (2001), who reported the use of biocontrol agents in combination for controlling nursery rot disease of black pepper.

Various organic preparations for plant growth and disease suppression have been suggested by local farmers. In the present study, jeevamrutha, egg lemon juice extract and fish jaggery extract were found effective on plant growth especially on healthy leaf production and stem girth. Sudhanshu et al. (2015) reported that jeevamrutha is considered to be a panacea for the prosperity of agriculture. Higher nutrient availability of the preparations and its better uptake might have resulted in increased leaf area, chlorophyll production and better photosynthesis activity. Our results are in accordance with Priyanka et al. (2019), Ramesh et al. (2020) and Meyyappan et al. (2021) who reported the influence of fish amino acid and egg amino acid on growth and yield of green gram, amaranthus and rice respectively. In addition to growth improvement, application of fish jaggery extract, jeevamrutha and egg lemon juice extract reduced the pathogenic infection on black pepper plants. The results confirm with the findings of Abbasi et al. (2003) who reported that weekly spraying of neem oil and fish emulsion reduced disease severity on the foliage of inoculated field-grown tomato and pepper plants. Karthika et al. (2017) reported the inhibitory effect of fermented egg-lemon juice extract on growth of *R. solani.* 40 to 100% inhibition of the mycelial growth of *R. solani*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Phytophthora colacasiae* and *Fusarium solani* using panchagavya has been reported by Sugha (2005). It may be attributed to the antimicrobial compounds or beneficial microorganisms and its metabolites produced during the preparation and fermentation of biopreparations along with the nutrients like calcium which might have contributed in defending the pathogenic invasion (Kai et al., 2014, Karthika et al., 2017).

4. Conclusion

The application of native isolates of antagonistic bacteria @ 2% combined with soil application of *Trichoderma* increased the plant height and number of leaves and jeevamrutha @ 10% increased the leaf area and chlorophyll contents whereas application of fish-jaggery extract @ 0.5% reduced the pathogen infection in black pepper cuttings. Hence, the indigenous biopreparations and native isolates of antagonistic microbes could be integrated in the healthy nursery management package for black pepper which is environmentally safe and economically viable.

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